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FLUORESCENCE INDUCTION STUDIES IN ISOLATED CHLOROPLASTS

III. ON THE ELECTRON-TRANSFER EQUILIBRIA IN THE POOL OF ELECTRON ACCEPTORS OF PHOTOSYSTEM II

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SUMMARY

The oxidation states of the primary and secondary electron acceptors of Photosystem II in isolated chloroplasts were measured simultaneously during their oxidation and reduction. The fluorescence yield and its variation were used as indicators for the oxidation states. An equilibrium treatment gives different contradicting results for different conditions: a low equilibrium constant ($K \approx 1$) for the reaction between the (reduced) primary carrier to (oxidized) secondary carriers when the system is photoreduced, and a high equilibrium constant ($K > 10$, sometimes $K > 100$) when the system is oxidized in the dark. This discrepancy is discussed in terms of two alternatives: (a) influence of the light, by secondary interactions, on reaction parameters; (b) possibility of two photoreactions in Photosystem II.

INTRODUCTION

Fast and reversible fluorescence yield changes in isolated chloroplasts are attributed to the state of the reaction center in Photosystem II. In the original quencher hypothesis of DUYSENS AND SWEERS[1], the electron acceptor part of the reaction center, called Q, is responsible for the fluorescence changes. The oxidized form Q quenches effectively the excitation energy on the pigment system, thus reducing fluorescence to a minimum, while the reduced form Q^- (or QH) cannot act as a quencher and thus in the transition $Q \rightarrow Q^-$ the fluorescence increases.

In previous studies it was shown[2] that strong light absorbed by isolated chloroplasts reduces pool electron carriers of Photosystem II. From the light requirement of the fluorescence rise, we were able to calculate[2] that a pool of electron acceptors equal to about 1/20 chlorophyll is reduced. From the fluorescence kinetics and from other pieces of evidence[2,3] it was assumed that the pool is composed of two parts acting in series that were called Q and P. Similar conclusions were also reached by MURATA *et al.*[4].

Our model failed, however, to explain the fast fluorescence rise in the presence

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Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)[4,5] and in very high light intensities[5,6]. In view of this a modification of the model was introduced by the methods of JOLIOT AND JOLIOT[7], and FORBUSH AND KOK[8] who postulated a small amount of primary quencher Q in equilibrium with a pool of secondary electron carriers A. The original work of JOLIOT AND JOLIOT[7] maintains that the main pool A is composed of two (about equal) parts, A_1 and A_2, reacting linearly (Fig. 1) and that mainly A_1 is in close equilibrium with Q ($K \approx 1$). FORBUSH AND KOK[8] explain the kinetic form of the fluorescence induction curve by assuming that both A_1 and A_2 react directly with Q (Fig. 1b) but with different rate and equilibrium constants.

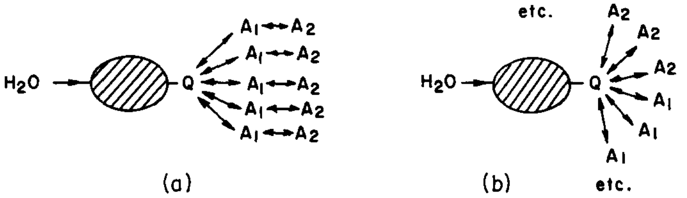

Fig. 1. Models for the electron transfer processes in the pool system. (a) Consecutive electron transfer from Q to A_1 and to A_2. (b) Parallel electron transfer from Q to A_1 and A_2.

One of the main reasons for introducing an equilibrium between the primary oxidant Q and the pool is that the general form of the fluorescence rise curve indicates a slow reduction of Q parallel to the reduction of the pool. If the reaction between Q and the pool is irreversible the form of the fluorescence rise curve would be completely different: no rise for most of the time when the pool is reduced, followed finally by a sharp rise which indicates the reduction of Q.

The assumption of the above equilibrium destroys our previous kinetic model[9] about the interaction between the two main parts of the pool in which we postulated an independent-chain first-order reaction sequence. The equilibrium necessarily introduces intercommunication between the chains.

In view of the above a systematic investigation of the equilibrium properties of the pool system was carried out by fluorescence methods. We start from the model of JOLIOT AND JOLIOT[7] and FORBUSH AND KOK[8] and assume the following equilibrium reactions (*cf.* Fig. 1):

$$Q^- + A_1 \rightleftharpoons Q + A_1^- \text{ equilibrium constant } K_1 \qquad \text{(i)}$$

$$Q^- + A_2 \rightleftharpoons Q + A_2^- \text{ equilibrium constant } K_2 \qquad \text{(ii)}$$

From the standpoint of equilibrium it is immaterial whether it is Q^- or A_1^- that react with A_2. A choice of any two independent formal equilibrium reactions is permissible. Therefore, our results do not distinguish between the models of Fig. 1.

In order to measure the above equilibria we brought the system into various degree of reduction or oxidation by either applying light for a given period or shutting off the light and giving a certain period of darkness. These procedures reduce or oxidize the pool ($Q + A_1 + A_2$) in rates much smaller than the rates of electron transfer within the pool system, which implies that the pool system is always very close to equilibrium.

The results reported here are not consistent with either schemes of Fig. 1. We did not obtain any simple equilibrium correlation between Q and the pool. In most of our experiments the results obtained from an analysis of the fluorescence induction curve differed markedly from those obtained from an analysis of fluorescence restoration (the back reaction of fluorescence induction) curves.

We do not aim in this stage to make a definite model to explain this discrepency, except for a few speculations that can serve as guide for further experimentation (*cf.* DISCUSSION).

When this work was completed, we were informed that similar experimental results were achieved by OWENS AND KOK[10] and also by P. JOLIOT AND P. BENNUN (personal communication).

METHODS

Chloroplasts were isolated from lettuce by standard procedure[11]. Fluorescence induction was measured by a similar technique as described previously[2]. Fluorescence light was selected by a very sharp interference filter peaked at 695 nm (half-band width = 3 nm).

Some experiments were carried at low temperature (—20°). In these experiments we followed the method of FISCHER[12] for maintaining and controlling low temperatures. The vessel was kept in a copper block, cut in such a way to permit irradiation and fluorescence. The block was put inside a glass dewar. The cooling was done by streaming liquid air through the copper block with the aid of a vacuum pump. This was regulated by a magnetic valve controlled by a thermoregulator (Wheelco). In order to prevent the reaction mixture from freezing, we used 30% glycerol in water as a reaction medium for the low temperature experiments. In this medium there was some change in the fluorescence kinetics, manifesting itself especially by the disappearance of the characteristic inflection[2], but no other drastic change from normal behaviour was noted. The full induction curve was obtained at low temperature after far-red irradiation at room temperature, followed by cooling in complete darkness.

Steady-state or momentary values for the oxidation state of Q were obtained by measuring the initial fluorescence yield at the onset of a strong standard light beam. (510–630 nm, 4 nanoeinsteins$\cdot$cm$^{-2}\cdot$sec^{-1} from a Brown Slide projector operated on d.c. line.) The oxidation state of the pool ($Q + A_1 + A_2$) was obtained similarly by estimating the area of the fluorescence induction curve of the same standard beam in comparison to the area of a standard curve obtained after preillumination by far-red light, which oxidizes all the intermediates between the two photosystems (far-red light was 724 nm, 5 nanoeinsteins$\cdot$cm$^{-2}\cdot$sec^{-1} from d.c. Xenon lamp).

Fig. 2 summarizes this method of calculation. Using the definitions shown in Fig. 2, we have:

$$q = \frac{Q_{\mathrm{ox}}}{Q_{\mathrm{total}}} = \frac{F_\infty - F_{\mathrm{i}}}{F_\infty - F_0} \tag{1}$$

according to a linear relation[2] or

$$q = \frac{(F_\infty - F_{\mathrm{i}})/(F_\infty - F_0)}{2 - (F_\infty - F_{\mathrm{i}})/F_\infty - F_0)} \tag{1a}$$

which is a simplification of an equation introduced by JOLIOT AND JOLIOT[7] assuming energy transfer in Photosystem II units with a yield of 0.5.

$$p = \text{oxidation state of the pool} = \frac{Q + A_1 + A_2}{Q_{\text{total}} + A_{1\,\text{total}} + A_{2\,\text{total}}} = \frac{S}{S_m} \tag{2}$$

Let us define the oxidation state of A_1 and A_2 by a_1 and a_2.

$$a_1 = \frac{A_1}{A_{1\,\text{total}}} \tag{3}$$

$$a_2 = \frac{A_2}{A_{2\,\text{total}}} \tag{3a}$$

Let us find the connection between the reduction state of Q and of the pool: If the fractions of total Q, A_1 and A_2 in the total pool are γ, α_1 and α_2, respectively, then it is obvious that:

$$p = \gamma q + \alpha_1 a_1 + \alpha_2 a_2 \tag{4}$$

From the equilibrium reactions, i and ii, we obtain:

$$\frac{[A_1^-]}{[A_1]} = K_1 \frac{[Q^-]}{[Q]} \tag{5}$$

$$\frac{[A_2^-]}{[A_2]} = K_2 \frac{[Q^-]}{[Q]} \tag{6}$$

Combining Eqns. 4, 5, and 6 we get an equation connecting the oxidation states of Q and the pool.

$$p = \gamma q + \frac{\alpha_1}{K_1\left(\frac{1}{q} - 1\right) + 1} + \frac{\alpha_2}{K_2\left(\frac{1}{q} - 1\right) + 1} \tag{7}$$

In order to evaluate the experimental results in terms of Eqn. 7 we notice that Eqn. 7 is composed of three terms of the same functional form[*]

$$\frac{1}{K_i\left(\frac{1}{q} - 1\right) + 1} \tag{8}$$

This function (8) is plotted in Fig. 5 for various values of K_i.

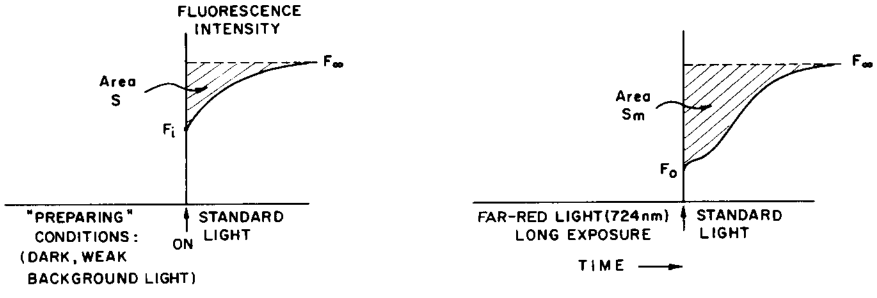

Fig. 2. Schematic representation of the procedure and the measurement of the fluorescence parameters. F_i, initial fluorescence yield; F_∞, maximal fluorescence yield; S, area above fluorescence curve limited by $F = F_\infty$ and $t = 0$. The right picture represents a standard full fluorescence curve with the parameters $F_i = F_0$ and maximal area $S = S_m$.

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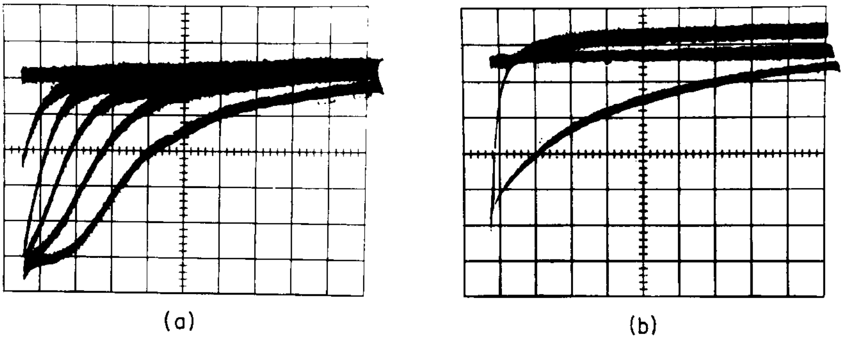
[*] Notice that the first term has also the same form if one puts $K_i = 1$.

RESULTS

p vs. q relation in the dark oxidation process

Fig. 3a shows fluorescence induction curves; the one with maximum area is obtained after 1 min exposure to far-red (724 nm) light, the others after various times in darkness following a previous standard exposure to strong green (510–630 nm) light. It is easily seen that in the beginning, the relaxation of fluorescence to F_0 in the



(a) (b)

Fig. 3. Oscilloscopic picture of the fluorescence induction curves obtained after different dark periods. (a) Room temperature dark times: 2.5, 5, 20, 60 sec. The "full" fluorescence induction is obtained after 1 min of far-red light. Reaction medium: 0.2 M sucrose, 0.01 M NaCl, 0.01 M Tricine (pH 7.5) and 8 μg/ml chlorophyll. Oscilloscope time scale: 0.5 sec/div. (b) −20°. The full induction is obtained after 1 min far-red at room temperature followed by cooling to −20°. The "partial" fast fluorescence rise is obtained after 10 min in the dark following the previous irradiation. Reaction medium is the same as in (a) except that the solvent is 30% (v/v) glycerol in water. Oscilloscope time scale, 1 sec/div.

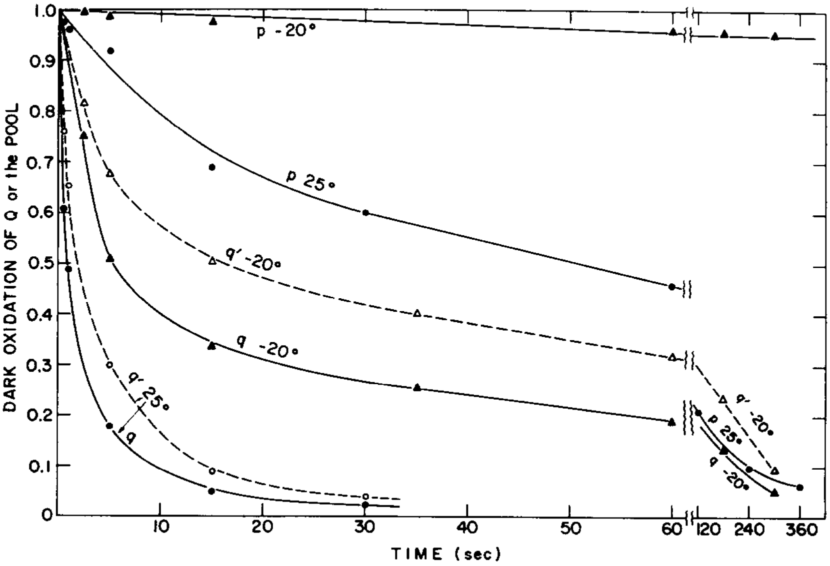


Fig. 4. Restoration kinetics of Q and the pool from the reduced to the oxidized state at room temperature and at −20°. q and q' refers here to the reduced state of Q, q is calculated according to the linear relation between fluorescence and q (Eqn. 1); q' is calculated according to Eqn. 1a. p refers to the reduced state of the pool. Conditions, the same as in Fig. 3.

darkness does lead to only small comparable relaxation of the area above the induction curve; the area develops only after a longer period in the dark.

Fig. 4 exemplifies the above result by a direct plot of the oxidation kinetics of Q and the pool for the restoration process of the fluorescence induction. This is a direct demonstration that the oxidation of Q is much faster than the oxidation of the pool so that the equilibrium constant between them must be higher than 1. This conclusion becomes even more obvious from the results of an experiment performed at —20°. At this temperature the reoxidation of the pool essentially stops, but the oxidation of Q proceeds in a considerable rate until Q is completely oxidized; the area above the induction curve when Q is completely oxidized is only about 1/20 of the maximum, corresponding to the amount of Q only (Figs. 3b and 4).

Fig. 5 shows theoretical plots of p *vs.* q assuming a contribution of only one term in Eqn. 7.

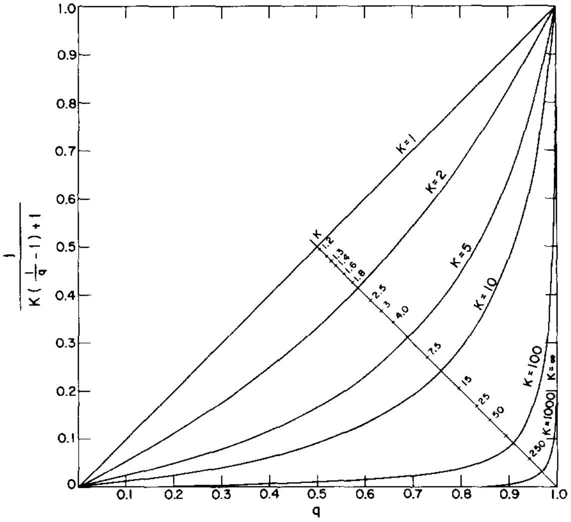


Fig. 5. Theoretical plots of $1/[K(1/q - 1) + 1]$ *vs.* q for various values of K.

In Fig. 6a or 6a′ Curves 1–4 show a few experimental examples of p *vs.* q in the dark calculated according to the linear Eqn. 1 (Fig. 6a) or the nonlinear Eqn. 1a (Fig. 6a′). (Compare with the theoretical curves of Fig. 5.) The most typical results fitted curves between 2 and 3. However, we noticed quite large deviations occurring from time to time, in some preparations, from the behaviour of Curves 2 and 3. In any case, the exceptions were quite rare (16 out of 20 experiments gave results similar to Curves 2 or 3). The exceptional behaviour may be caused by some unknown intrinsic difference in preparation. Nevertheless, taking the various curves together, it appears that apparently there exists no definite correlation between the oxidation state of Q and the pool during darkness.

An exact analysis of these curves should take into account the possibility of contribution of the three terms according to Eqn. 7. First there is the linear term γq where γ, the fraction of Q in the pool, is estimated[8] to be about 1/20. The intercept

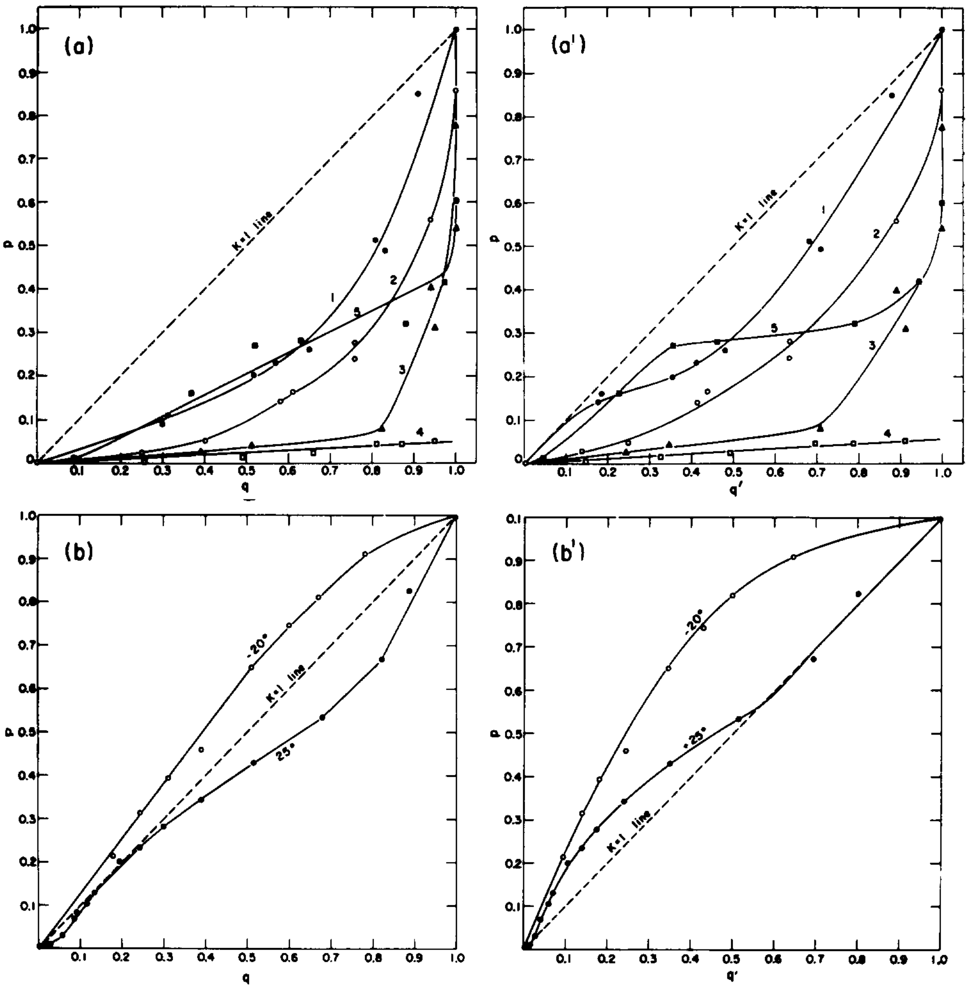


Fig. 6. The oxidation state of the pool (p) *vs.* the oxidation state of Q (q) for various experiments. (a, a′) curves 1–3, p *vs.* q during dark reoxidation at room temperature, samples from about 20 experiment. Most of the experiments fitted curves between 2 and 3. Reaction mixture was 0.2 M sucrose 0.01 M NaCl and 0.01 M Tricine (pH 8) for Curve 1, and the same but without sucrose (pH 7) for Curves 2 and 3. In these experiments the pH was varied from 6.5 to 8.5 without any systematic influence on the behaviour, also the presence or absence of sucrose was not critical. Curve 4, dark reoxidation at −20°, conditions as in Fig. 4. Curve 5, steady-state in green (510–630 nm) light for various light intensities (in the range 0.1–40 nanoEinsteins·cm^{-2}·sec^{-1}); reaction mixture as in 2. (a Calculations according to the linear relation (Eqn. 1; (a)′ calculations according to the non linear relation (Eqn. 1a). (b, b′) p *vs.* q during reduction in the light (direct analysis of the fluorescence induction curve). Room temperature and −20°, as indicated; conditions as in Fig. 3. (b) Calculations according to the linear relation (Eqn. 1); (b′) calculation according to the non linear relation (Eqn. 1a).

of p *vs.* q with the $q = 1$ axis gives, in an appropriate way, the contribution α_2 of the term (s) containing very large K[*].

$\alpha_2 = 1 -$ intercept at $q = 1$

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[*] This estimate depends of course on the accuracy of the results. For very accurate results this intercept gives the contribution of the term with $K = \infty$ exactly. However since even with $K = 100$ the curve approaches the axes $p = 0$ and $q = 1$ considerably, we estimate that the very large K in our case means K in order of magnitude of about 100 or more.

From this we may also estimate α_1 from $\alpha_1 + \alpha_2 + \gamma = 1$.

Such an analysis is summarized in Table I.

TABLE I

ANALYSIS OF THE PARAMETERS OF p *vs.* q CURVES

γ is the fraction of Q in the pool. In the cases marked * it is assumed to be 0.05. In other cases it is calculated from initial slope. α_1 is the fraction of the pool having a small measurable equilibrium constant which is indicated in the column K. α_2 is the fraction of the pool having a very large (>100) equilibrium constant which is not indicated. In the case that no small equilibrium constant exists we put the values of $\alpha_1 + \alpha_2$ together, *e.g.* in Fig. 6, Curve 3, *plus* an estimation for K. K is the smallest equilibrium constant for the part of the pool.

Fig.	*Curve*	γ	α_1	α_2	K	*Remarks*
6a	1	0.05*	0.95	0	~ 5	
6a′		—	0.95	0	~ 2	No exact fit
6a	2	0.05*	0.80	0.15	~ 10	
6a′		0.05*	0.80	0.15	~ 5	
6a	3	0.09	0.91		~ 50–100	
6a′		0.12	0.43	0.45	~ 30–50	
6a	4	0.05	0.95		> 1000	
6a′		0.06	0.94		> 1000	
6a	5	0.05*	0.45	0.50	1	
6a′		—	—		—	No fit
6b	25°	0.05*	0.95	0	~ 1	No exact fit
6b′		0.05*	0.95	0	~ 1	No exact fit
6b	−20°	0.05*	0.95	0	~ 0.6	No exact fit
6b′		0.05*	0.95	0	~ 0.25	
7	Dark				~ 100	
	Light				~ 1	

p vs. q in the light during induction

Figs. 6b and 6b′ show the relation between p and q in the light during the fluorescence induction. For this experiment we used rather low intensity green (510–630 nm) light in order not to perturb the (assumed) equilibrium, but not too low in order to achieve a nearly complete reduction of the pool. We see that the plot p *vs.* q does not follow accurately the form predicted in Fig. 5 but is only roughly close to the line representing $K = 1$ (compare with Figs. 6a and 6a′). This experiment was also repeated at −20° giving essentially the same result ($K < 1$). The results of this experiment are also summarized in Table I. Fig. 7 directly demonstrates, in a concluding experiment which was performed on a single sample, the "hysteresis" in the p *vs.* q curve: different relations during the photoreduction of the pool and the dark oxidation, in agreement with Fig. 6.

p vs. q during steady state in different light intensities

Curve 5 in Fig. 6a (or 6a′) shows the p *vs.* q relation for different intensities of green light (510–630 nm) preillumination. By reducing the green light intensity a steady state is achieved where Q and the pool may be partly oxidized until almost fully oxidized at very low intensity. The results of this experiment are also summarized in Table I.

We have observed an obvious discrepancy in the relation of p and q between the dark when the pool slowly oxidizes, and the light when the pool slowly reduces, a high equilibrium constant in the former case compared to low values in the dark.

We have calculated that the deviation from equilibrium caused by the external driving processes of reduction and oxidation of the pool are negligible and cannot account for this discrepancy.

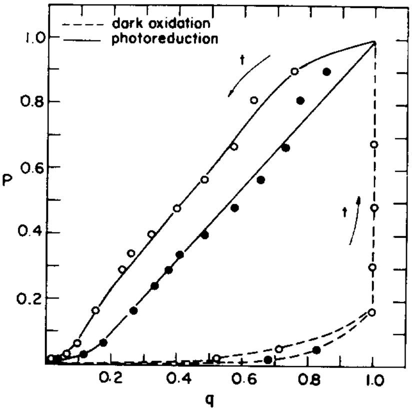

Fig. 7. p vs. q for a single sample in the light and the dark. During photoreduction there is a transition from point (1:1) to (0:0) following the solid line (●, linear relation, Eqn. 1; ○, non-linear relation, Eqn. 1a). During dark there is a back transition following the dashed line. Reaction mixture: 0.01 M NaCl, 0.01 M Tricine (pH 7.5), 10 µg/ml chlorophyll. Green light (510–630 nm) of 10 nanoEinsteins·cm^{-2}·sec^{-1}.

DISCUSSION

The results of the experiments carried in the dark are most pertinent for conclusions about equilibrium properties since in the absence of any trustable model, one can in general assign the results of experiments carried out in the light to a fast build-up of some steady state (but not equilibrium).

From most of the experiments carried out in the dark, we conclude that if there is a reaction between Q^- and the pool, the equilibrium constant is large (10, sometimes 100). On the other hand, the fact that the results have such a big spread from sample to sample points to the conclusion that Q and the pool are oxidized in different ways and rates, and no direct reaction exists between them.

This conclusion is strengthened by the remarkable change of equilibrium constant to about 1 during the fluorescence induction. This indicates that only with light a correlation is built between Q and the pool. To some extent this is also true for the steady state in the light where at least a considerable part of the pool shows an equilibrium constant close to 1 with Q.

One possibility is to make an *ad hoc* hypothesis and to postulate a gross change of (dark) reaction constants influenced by a triggering action of the light. One can visualize that in the first moment during light there is a molecular conformational change which causes Q to approach closely the A molecules of the pool, allowing a reaction between them. In the dark a reverse movement separates them and the electron transfer process between them is inhibited. However, this explanation suffers from the inability to check such a fast molecular conformational change directly. Most light-dependent macroscopic conformational changes essayed by such methods as light scattering are much slower then required in this case. In our case we expect that the system adapts itself to the changing condition in much less time than the measurement time, *i.e.* about 0.1 sec. Typical conformational change times are usually in the order of 10 sec or more.

It is interesting to note that similar equilibrium properties were observed on other e^- carrier systems. For example, measuring the system $Q \rightleftharpoons P_{700}$ JOLIOT *et al.*[13] found a small equilibrium value (K 3–10) in the light, compared to much higher values in the dark. This was also noted in a recent work by MARSHO AND KOK[14] on the equilibrium cytochrome $f \rightleftharpoons P_{700}$. The last work independently also arrives to our above assumption that light may change kinetic parameters by some secondary interactions. One possibility of accounting apparent equilibrium properties is the independent electron transfer chain concept[15], but this is not applicable directly to our case since most of our measurements are not carried out during the steady state.

Ignoring for a moment the possibility that the light has a triggering effect, the second possibility to consider is that there exists another light reaction besides the one which reduces Q, which establishes the correlation between Q and the pool during light.

We therefore make another *ad hoc* hypothesis and introduce the idea of two photoreactions instead of one, which both bring about the reduction of the pool. Since the pool is known to be oxidized by Photosystem I we must assume that the two photochemical acts now postulated belong to Photosystem II and are called

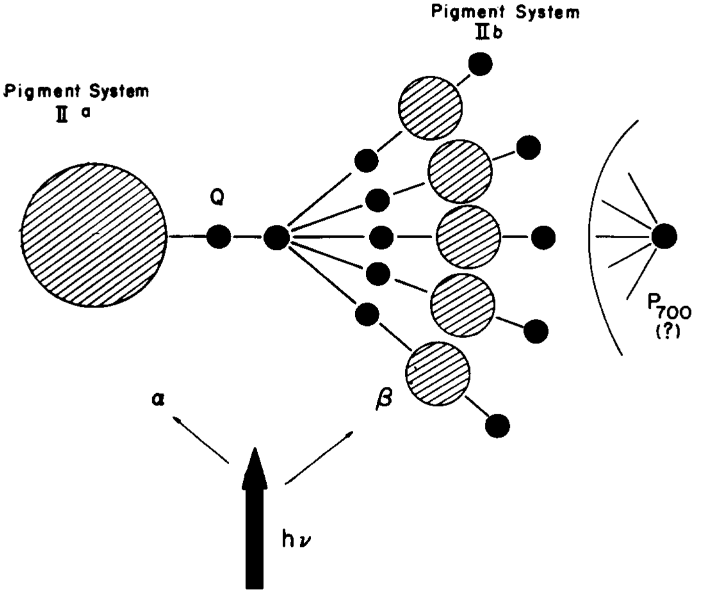

Fig. 8. A possible theoretical model for the arrangement of two photoreactions in Photosystem II. Black spots, electron carriers; striped spots, pigment systems. The total number of chains is not indicated and should be of the order of 10. The pool is composed of about two equal parts to the right and the left of Photosystem IIb. α and β are light distributions to Photosystems IIa and IIb from the total light reaching Photosystem II. P_{700} may be included as a terminal electron acceptor to the pool (*cf.* text).

accordingly Photosystem IIa and IIb. The same idea was expressed by KNASS AND ARNON[16], although for completely different reasons.

Fig. 8 shows a possible model for the arrangement of the pool system with two photoreactions. A theoretical calculation on this system was made, allowing different distribution of light to Photosystems IIa and IIb. It is shown that theoretical fluorescence induction curves can be reproduced and show relatively slow reduction of Q together with slow reduction of the pool (Fig. 9). p *vs.* q curves for these theoretical plots are also shown in Fig. 10. Unfortunately, not all experimental induction curves

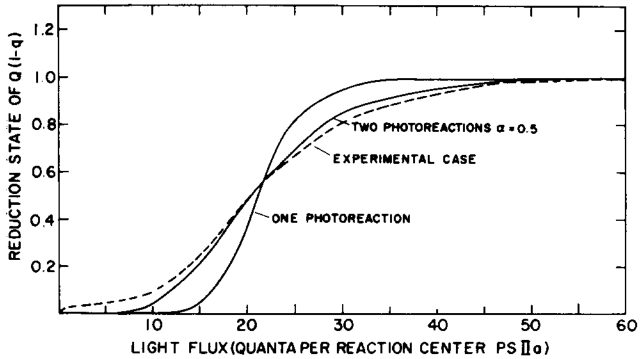

Fig. 9. Theoretical fluorescence induction curves: Model Fig. 8 with $\alpha = 0.5$; Model of one photoreaction with pool/Q = 20 calculated by omitting light reaction IIb in Fig. 8 and taking into account the possibility of dark transfer of electrons (*cf.* pool = 20 in Fig. 10). One experimental case (PS IIa, Photosystem IIa).

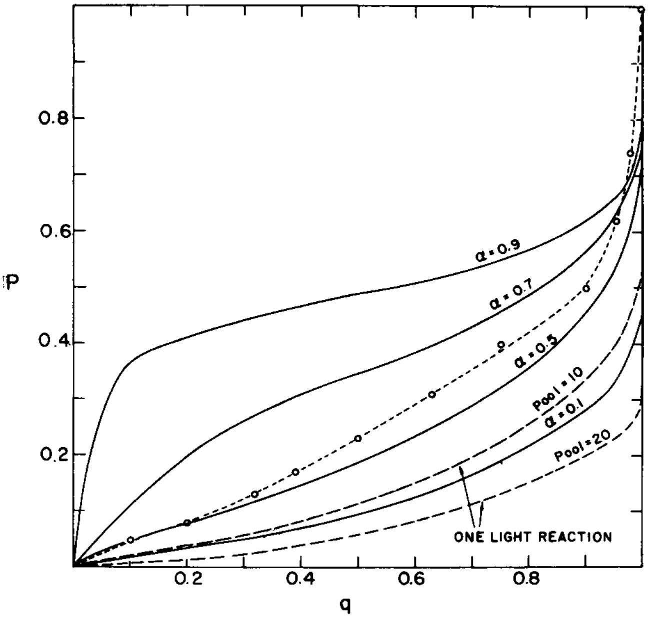

Fig. 10. Theoretical p *vs.* q curves calculated according to the model of Fig. 8 (solid lines). The dashed lines indicated by pool = 10 and pool = 20 are calculated for a similar model but with only one photoreaction: for pool = 10 both Photosystem IIb and its e^- acceptors are omitted; for pool = 20 only Photosystem IIb is omitted and dark transfer of electrons is assumed. ○---○ corresponds to an analysis of an experimental induction curve (*cf.* Fig. 9).

show good fit to these theoretical curves except for a few examples, one of which shown in Fig. 10. It fits a case where there is about equal distribution of light to Photosystems IIa and IIb. We can only say that at this moment the idea of two photoreactions at Photosystem II "corrects" the theoretical curves in the right direction but not sufficiently. (Compare the curve for one photoreaction and pool = 20 in Fig. 10.)

In the dark no correlation of q and p must exist; Q and pool may be oxidized in completely noncorrelated ways.

The theoretical calculations are based on the following scheme, using Fig. 8: At any moment there exist several species with the general structure of Fig. 8 but with different redox states. We label the redox state of the pool between the photosystems by an index i ($i = 0.1 \ldots . N+2$) and the redox state of the pool to the right of Photosystem IIb by an index j ($j = 0.1 \ldots . N$). N is the number of chains originating from Q. We took $N = 10$ so that the pool/Q ratio becomes 22 according to Fig. 8. Assuming an even distribution of light within similar aggregates, and no energy transfer, we may write the following differential equation for the molar fractions $f_{i,j}\,(t)$ of the species (i, j):

$$\frac{\mathrm{d}f_{i,j}}{\mathrm{d}\lambda} = -\alpha\rho(i)f_{i,j} - \beta\sigma(i,j)f_{i,j} + \alpha\tau(i)f_{i-1,j} + \beta\mu(i,j)f_{i+1,j-1} \tag{9}$$

where $\alpha, \beta \equiv$ fractions of light absorbed in Photosystems IIa and IIb respectively. ρ, σ, τ, μ are given functions of the indices i and j, defined as follows:

$$\begin{cases} \rho(i) = 1 & \text{for } i < N+2 \\ \rho(i) = 0 & \text{for } i = N+2 \end{cases}$$

$$\begin{cases} \tau(i) = 0 & \text{for } i = 0 \\ \tau(i) = 1 & \text{for } i > 0 \end{cases}$$

$$\begin{cases} \sigma(i,j) = \dfrac{i(N-j)}{N^2} & \text{for } i \leqslant N \text{ all } j \\ \sigma(i,j) = \dfrac{N-j}{N} & \text{for } i > N \text{ all } j \end{cases}$$

$$\mu(i,j) = 0 \qquad \text{for } i = N+2 \text{ all } j$$

$$\mu(i,j) = 0 \qquad \text{for } j = 0 \text{ all } i$$

$$\mu(i,j) = \frac{(i+1)(N-j+1)}{N^2} \qquad \text{for } i \leq N-1 \text{ and } j > 0$$

$$\mu(i,j) = \frac{N-j+1}{N} \qquad \text{for } N-1 < i < N+2 \text{ and } j > 0$$

λ is the normalized light input in terms of $I \cdot t$ (quanta/reaction centers).

The above equation accounts for the reduction of a species (i,j) by Photosystem IIa to $(i+1,j)$ with a yield $\alpha \cdot f_{i,j}$, and the oxidation of (i,j) to $(i-1, j+1)$ with a yield $\beta\sigma\,(i,j)\,f\,(i,j)$. All reactions are assumed to be essentially first order for any given species except for introduction of the factor $\sigma\,(i,j)$ which is a probability factor for the occurrence of a reduced donor and oxidized acceptor for Photosystem IIb on the same chain. The factor μ is the same as σ except for an index shift. The factors ρ and τ were introduced for convenience to allow for the change in the form of the equations with the extreme values of the index i.

The differential Eqn. 9 was solved numerically by a computer. Fig. 9 give an example of a solution to Eqn. 9 for $\alpha = 0.5$ and also for the case of only one photoreaction with pool/Q ratio = 10 (model Fig. 8 with omission of Photosystem IIa and its e^- acceptors) and pool/Q ratio = 20 (model Fig. 8 with omission of Photosystem IIa but not the e^- acceptors).

The above scheme is of course highly speculative, and even more so the particular one in Fig. 8, which is just one example of the many more possible schemes of two photoreactions in Photosystem II. We will make an attempt to check other possibilities in further work.

Fig. 8 has certain symmetry with regard to Q and P_{700} if one also includes P_{700} into the picture and adds it as a terminal e^- acceptor to the pool. It may be noted that the same dilemma of the equilibrium between Q and the pool was found also with P_{700}, and the pool (in far-red light P_{700} is oxidized slowly, more or less in a parallel manner to the pool, indicating a small equilibrium constant; in the dark the pool is oxidized and P_{700} reduced in contrast to such an equilibrium[17]). The idea of two photoreactions within the pool system may also explain this last phenomenon in the same way as it explains the photoreaction of Q.

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